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Direct Agglutination Test for Diagnosis of *Toxoplasma* Infection: Method for Increasing Sensitivity and Specificity

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A method that increases the sensitivity and specificity of the direct agglutination (AG) test for diagnosis of *Toxoplasma gondii* infection is described. Qualitative results in the Sabin-Feldman dye test (DT) and AG test were in excellent agreement (98%). Differences in titers between these two tests often related to the length of time the individual was infected. The AG test titer was most often lower than the DT titer during acute (recent) infection; the AG test titer was most often higher than the DT titer in older or chronic infection. If the AG test antigen described here can be made available, the AG test would be ideal for use as a screening test and would provide a simple and inexpensive means for the surveillance of seronegative women during pregnancy and for detection of seroconversions.

At present, the diagnosis of acute acquired infection with *Toxoplasma gondii* depends on serological test results. A number of problems associated with available methods for serodiagnosis of this infection have been an impetus to search for alternative methods. The routine methods presently available to physicians in practice are often expensive to perform, time consuming, not readily adaptable to screening programs, or not sensitive enough to be useful in early diagnosis of the infection (e.g., the hemagglutination test).

The toxoplasma agglutination (AG) test was first described by Fulton et al. (5-8). This method uses whole killed organisms and is now routinely performed in France with an antigen that has been prepared as described by Ardoin et al. (1) and that is commercially available (bioMérieux, Charbonnières-les-Bains, France). The method is very simple and useful but as presently used has two drawbacks. First, it lacks sensitivity; the titer in the AG test is usually much lower than that in the dye test (DT) or the conventional immunofluorescent-antibody (IFA) test. As a consequence, some sera that are positive in the latter two tests are reported as negative in the AG test. Second, it lacks specificity; some sera that are negative in the DT and IFA test are reported as positive in the AG test (2). This has been shown to be due to the binding of normal immunoglobulin M (IgM) ("natural IgM antibody") to the surface of the parasite (3).

We describe here a method for preparing antigen which increases the sensitivity of the AG test and a method for suppressing nonspecific agglutination by the use of a buffer containing 2-mercaptoethanol (2ME). When the AG test is modified by these two methods, its specificity and sensitivity parallel those of the DT. The technique and the reading are so simple and accurate that, if the antigen is made available, the modified AG test method would be convenient for laboratories that perform serology only occasionally as well as for those that perform large-scale surveys.

MATERIALS AND METHODS

Preparation of the antigen. The antigen is prepared using the RH strain of toxoplasma cultivated along with mouse TG 180 sarcoma cells in the peritoneal cavities of mice. As many as 2×10^8 to 5×10^8 toxoplasma per mouse may be obtained with this method. This is approximately 10 times more than can be obtained when the conventional method of inoculating toxoplasma alone into the peritoneal cavities of mice is used.

Mice inoculated with the mixture of sarcoma cells and parasites develop an exudate containing both sarcoma cells and parasites, the proportion of which depends on the ratio of parasites to sarcoma cells inoculated and on the time elapsed after inoculation of the mixture. If one examines these exudates microscopically (400×, phase contrast), six different stages may be noted. In stage I, most cells are not infected, and those few (one in every two or three fields) that are infected contain only a few parasites. In stage II, 5 to 10% of cells are infected, each containing only a few parasites. In stage III, approximately half of the cells are infected, and most of these contain only a few parasites. A few cells are heavily infected. There are few extracellular toxoplasma. In stage IV, almost every

cell is heavily infected and close to disruption. Extracellular parasites are present but are few when compared with the tremendous numbers of intracellular organisms. In stage V, many heavily parasitized cells that are close to disruption are still present, and a great number of free toxoplasma that appear morphologically normal (alive) are observed. In stage VI, all sarcoma cells have been disrupted, and there is a tremendous number of free parasites. Many of the organisms are obviously dead, agglutinated, or undergoing lysis.

To obtain a satisfactory antigen for the AG test (and for the IFA test or DT as well), the following two requirements should be closely adhered to. (i) Exudate must be harvested when it is in stage IV or V; stages I, II, III, and VI should be discarded. (ii) Mice should be inoculated less than 72 h before their exudate is harvested; the optimal time is 48 h. These two requirements are difficult to fulfill if mice are inoculated with uninfected sarcoma cells mixed with toxoplasma obtained from mice that had been inoculated with parasites alone. Mice inoculated with this mixture usually die within 4 days (as they do when they have been inoculated with the RH strain of toxoplasma alone), and the infection in the sarcoma cells will not have reached the optimal stage by this time. It is for this reason that a two-step procedure has been developed.

The RH strain of toxoplasma is maintained by serial intraperitoneal transfer every 2 to 3 days. (For the RH transfer, 0.02 ml of 2- to 3-day exudate is inoculated intraperitoneally per mouse.) The sarcoma cells are maintained by intraperitoneal transfer every 10 to 12 days. (For the sarcoma 180 cell transfer, 10- to 12-day exudate is sedimented and 0.1 ml of the sedimented cells is inoculated intraperitoneally per mouse.) Two kinds of transfer are performed successively to prepare the antigen. In transfer A, mice are inoculated with a mixture of sarcoma cells and toxoplasma (2 ml of sarcoma cell exudate [10 to 12 days] is mixed with the entire ascitic fluid from a single mouse inoculated with the RH strain 2 to 3 days earlier). The mixture is then centrifuged, and the entire sediment is inoculated intraperitoneally into one mouse. (This is most easily done by injecting the 2 ml of sarcoma cell exudate intraperitoneally into the RH-infected mice and then immediately withdrawing the entire volume of exudate from the peritoneal cavities. The entire volume can then be injected into a single mouse. Just before injection, the fluid should be examined microscopically to check for bacterial contamination.) After 2 days, the exudate is examined microscopically (400×, phase contrast) for the absence of bacteria and for the stage of sarcoma cell infection. In transfer B, exudate from transfer A is mixed with the appropriate number of uninfected sarcoma cells. For this purpose, both the exudate obtained from transfer A mice (infected cells) and the exudate from a mouse injected with sarcoma cells only (uninfected cells) are centrifuged for 5 to 10 min at 500 to 600 × *g*. The sedimented cells are then mixed. The optimal ratios of infected cells to uninfected cells differ, depending on the stage of the exudate from the transfer A mice (Table 1). Two-tenths milliliter of the appropriate mixture of infected and uninfected cells is then inoculated intraperitoneally into fresh mice. Two days later, the peritoneal exudate will usually have reached stage IV or V and is therefore ready to be harvested for the preparation of antigen.

The next step is to free the toxoplasma from the infected sarcoma cells without damaging the organisms. During this procedure, the toxoplasma are exposed to trypsin. Cells, when heavily infected, are easily destroyed by trypsin treatment. The increased sensitivity of the antigen prepared by this method, compared with that commercially available, is not due to the effect of trypsin on toxoplasma since antigen preparations with organisms mechanically liberated from cells are just as sensitive. If the period of trypsinization is too long, however, or if the concentration of the enzyme is too high during trypsinization, the toxoplasma will have decreased value as antigen even though they retain their morphology. It is for this reason that the procedure must be interrupted as soon as most of the toxoplasma have been released from the sarcoma cells.

The sediment of the pooled stage IV and V exudates is resuspended in phosphate-buffered saline, pH 7.2 (PBS), containing 0.05% trypsin and incubated in a water bath at 37°C under continuous agitation. Portions are examined microscopically every 5 min for the disruption of cells. As soon as the cells are disrupted, the suspension of parasites is centrifuged for 10 min at 500 to 600 × *g*, the supernatant is discarded, and the sedimented parasites are resuspended in PBS and centrifuged again. After the second centrifugation, parasites are suspended in Formalin that has been diluted 1:5 in PBS (6% formaldehyde solution). The parasites are kept overnight in Formalin. The following day (at least 16 h after suspension in Formalin), they are centrifuged and resuspended in PBS. The sediment is washed in PBS three times to remove both cell debris and the formaldehyde. The parasites are then suspended in an alkaline buffer (pH 8.7) which contains 7.02 g of NaCl, 3.09 g of H_3BO_3, 24 ml of 1 N NaOH, 4 g of bovine plasma albumin (fraction V), and enough distilled water to bring the volume to 1 liter. (This formula is also used as the alkaline buffer in the hemagglutination inhibition test for rubella antibodies, which is commercially available. In the hemagglutination kits, it is recommended that the buffer be adjusted to pH 9, but it is usually pH 8.7.) Sodium azide (0.1%) is added as preservative.

The concentration of Formalin is important. Parasites should retain their normal crescentic shape. When insufficiently fixed, a less sensitive antigen is obtained.

The pH is critical for obtaining a reliable AG test. Spontaneous agglutination (sedimentation as a carpet) occurs when the pH is acid. The final pH in the wells should be slightly greater than 8 (8.2 to 8.4). In the technique described by Peloux et al. (10), sera are diluted in alkaline buffer and the antigen suspension is neutral. It was more convenient for us to have the initial dilution of sera in a buffer (PBS) that will work for the DT, AG test, and IFA test as well. As a consequence, we suspend the antigen in the alkaline buffer.

When the suspension is checked microscopically, it is usually found to consist almost entirely of free

TABLE 1. *Optimal ratios of infected and uninfected cells for transfer B*[a]

Stage of infection	Vol of infected cells (undiluted sediment)	Vol of uninfected cells (undiluted sediment)
I	1	1
II	1	2
III	1	4
IV	1	16

[a] See Materials and Methods.

parasites. When a few sarcoma cells are still present, they are eliminated by differential centrifugation (5 to 10 min at about 15 × *g*). If fibrinous coagulated material is present, it can be removed by filtering the suspension through a small piece of cotton wool. The concentration of parasites is adjusted to give optimal results when tested with sera known to be positive in the DT. The suspension is kept at 4°C (not frozen). It retains its antigenic properties for over 1 year. To adjust the suspension of toxoplasma to the optimal dilution for testing, a series of dilutions of a heavy suspension (stock suspension) of parasites is prepared and tested by block titration against the World Health Organization (WHO) standard (or a secondary standard) of positive toxoplasma antiserum (14). As a rule, a maximum agglutination pattern of +++ (an even carpet of organisms) is obtained up to an initial dilution of 1:4,000 of the WHO standard, which corresponds to a final concentration of 0.125 IU per ml in the reaction well. Readings of ++ and + are obtained with dilutions of 1:8,000 and 1:16,000 (0.063 and 0.031 IU/ml). A doubtful or negative (button) result is obtained with dilutions of 1:32,000 (0.016 IU/ml). The dilution of the stock suspension used as antigen is that for which the results are clearest in this range of values. It usually contains approximately 20,000 organisms per μl.

Performance of the test. It is not necessary for sera to be heat inactivated. The results are the same with heat-inactivated and non-heat-inactivated sera. The test is performed in microtiter plates with round (U-shaped) wells. We prefer U- to V-shaped wells since the former allow easier reading with suspensions containing fewer parasites (approximately 20,000 instead of 100,000 parasites per μl as described by Peloux et al. [10]) than do V-shaped wells. For dilutions, we used volumes of 50 μl since this working volume is more accurate than 25 μl.

Fifty microliters of 0.2 M (14 ml/liter) 2ME diluted in PBS is distributed in each well. It is important that the solution of 2ME be fresh (no more than 1 month old) and kept in a dark flask at 4°C. Twofold dilutions of sera are then performed, starting with 1:20. Sera known to be strongly positive in other serological tests should be tested, starting with a higher dilution (1:500, for instance). If screening is performed before titration, two dilutions (i.e., 1:20 and 1:500) should be tested to avoid false-negative results due to the prozone phenomenon. After all sera have been diluted, 50 μl of antigen is distributed in each well. The distribution of antigen may be performed immediately or may be delayed up to a few hours; this does not modify the results. The plates are agitated to allow for complete mixing of contents of the wells and then incubated at 32°C overnight.

The pattern of agglutination and sensitivity of the test are greatly modified when working at different temperatures. Incubation in the cold (4°C) results in increased titers and in spontaneous agglutination in negative controls. Caution must be exercised when the plates are incubated at room temperature overnight if there is no temperature control. It is for this reason that incubation in an incubator is recommended. We use 32°C. Care must be taken to prevent drying of the plates. Plates may be read at 16, 24, 48, or even 72 h without significant change in the titers as long as the wells have not dried.

The plates are read against a black background with a lateral light and are read by pattern. A smooth button at the bottom of the well is recorded as negative (0). A complete carpet is recorded as positive (+++). Intermediate readings from ± (doubtful) to ++ are also noted. In serial twofold dilutions, two or three tubes are usually noted to be ++ to ±, between definitely positive (+++) and completely negative (0).

The results are expressed as the reciprocal of the final dilution of serum. Because the sensitivity of the antigen is remarkably consistent, the titers may also be expressed in IU, as compared with the WHO standard. The last +++ well contains about 0.125 IU/ml. If, for instance, the last +++ well contains a 1:20 dilution of serum, the titer of the undiluted serum would be about 2.5 IU/ml.

The Sabin-Feldman DT was performed by a micromodification of the technique previously described (11).

RESULTS

Studies in Paris. The technique described for the AG test is used along with the DT and IgM-IFA test for routine examination of all sera tested for toxoplasma antibodies. A total of 640,000 consecutive sera have been examined with these three tests. Sera were first screened at a dilution of 1:20 and 1:800 in the DT and AG test. (It is important to note that it is necessary to screen with two dilutions, one low and one high, since a prozone phenomenon is often observed in strongly positive sera.) Sera positive at 1:800 were further examined in twofold dilutions from 1:800 to 1:102,400; when necessary for diagnostic purposes, sera positive at 1:20 were also examined in twofold dilutions from 1:20 to 1:2,560. When a discrepancy was noted in the screening procedure between results obtained in the two tests, sera were examined again in sixfold dilutions beginning with 1:10. In addition to routine testing of sera from patients, both the DT and AG test have also been used for comparison of the WHO standard serum for toxoplasma antibody to secondary standards. Examples of results of these procedures are shown in Tables 2 to 7.

The qualitative correlation between negative

(<1:20) and positive (≥1:20) results in the AG test and DT in 2,000 consecutive samples of serum is shown in Table 2. Both tests agreed in 98.4% of the sera. In 0.15%, the DT was positive and the AG test was negative; in 1.45%, the reverse discrepancy was noted.

Results of three separate examinations of the WHO standard for toxoplasma antibodies are shown in Table 3. The mean DT titer was 1:8,900, which corresponds to a concentration of 0.11 IU/ml. The mean AG test titer was 1:12,600 (0.08 IU/ml). (With most batches of antigen, a +++ reaction contains about 0.125 IU/ml and a + or ++ reaction contains about 0.06 IU/ml.)

The results of 100 consecutive examinations of the same positive control serum are shown in Table 4. The mean titer in the DT was 1:10,600, with no result greater than 1:25,600 or less than 1:3,200. The mean titer in the AG test was 1:13,810, with no result greater than 1:25,600 or less than 1:3,200. The results (in twofold dilutions) of the DT and AG test in 1,000 sera that were positive at 1:800 in the screening test are shown in Table 5, and results of 1,000 sera that were positive at 1:20 and negative at 1:800 in the screening test are shown in Table 6. In Table 7 are the results obtained in sera from 200 selected cases of recent acute infection. These samples were either the first positive sera obtained from pregnant women with previously negative serology who were routinely tested every month or the results of sera drawn from patients with clinical toxoplasmosis in whom the second sample of serum taken at a later date showed a significant increase in titer.

Studies in Palo Alto. Sera tested in these studies were chosen at random from groups of sera found to be negative or positive at various titers in the DT. In addition, sera from known cases of chronic infection and from cases of acute toxoplasmosis were used; these sera were obtained from a bank of specimens kept in the Palo Alto laboratory.

Table 8 shows the quantitative correlation between the DT and AG test titers in 202 samples of sera tested in Palo Alto. Twenty-nine of the sera gave results that were negative in both the DT and the AG test. One hundred and sixty-one sera were positive in both tests, and one serum was negative in the DT but positive in the AG test at a titer of 1:20.

TABLE 2. *Qualitative correlation of results in the DT and AG test in 2,000 consecutive sera*

DT	AG test		Total
	Negative, <1:20	Positive, ≥1:20	
Positive, ≥1:20	3 (0.15)[a]	1,164 (58.2)	1,167
Negative, <1:20	804 (40.2)	29 (1.45)	833
Total	807	1,193	2,000

[a] Numbers in parentheses are percentages.

DISCUSSION

The AG test first described by Fulton and Turk in 1959 (7) did not gain extensive use since it was difficult to obtain enough toxoplasma organisms for the AG test antigen. The technique described by Ardoin et al. in 1967 (1) overcame this difficulty and led to commercial production of the antigen. Once the antigen became commercially available, numerous studies which mainly emanated from France were performed, and many of the results have been published as part of a symposium held in Lyon in 1975 (4). From these studies, it appeared that the AG test titers were usually markedly lower than those obtained with the DT or IFA test and that "negative" results were often obtained in DT-positive sera. The occurrence of false-

TABLE 3. *Results of three examinations of the WHO standard for toxoplasma antibodies (1,000 IU/ml) in the DT and AG test*

Test	Date of assay			Geometric mean
	7/5/79	9/19/79	2/8/80	
DT				
Reciprocal of 50% endpoint dilution	6,000	10,000	12,000	8,900
Concn of antibody at this dilution (IU/ml)	0.16	0.10	0.08	0.11
AG test				
Reciprocal of last positive (+++ or ++) dilution	16,000	16,000	8,000	12,600
Concn of antibody at this dilution (IU/ml)	0.0625	0.0625	0.125	0.08

TABLE 4. *Results of 100 examinations of the same positive control serum in the DT and AG test*

Test	Titer[a]								Total	Geometric mean titer[a]
	800	1,600	3,200	6,400	12,800	25,600	51,200	102,400		
DT			1	26	72	1			100	10,600
AG test			1	4	79	16			100	13,810

[a] Reciprocal of dilution.

TABLE 5. *Quantitative correlation of titers in the DT and AG test in 1,000 sera with high antibody titers*

DT titer[a]	AG test titer[a]								Total	Geometric mean titer[a]
	800	1,600	3,200	6,400	12,800	25,600	51,200	102,400		
102,400										
51,200						1	[5]		6	45,500
25,600				4	8	[12]	13	2	39	26,070
12,800			7	18	[28]	26	14	4	97	16,300
6,400		11	35	[53]	37	23	9		168	7,950
3,200	10	34	[52]	28	35	31	4		194	5,521
1,600	31	[86]	63	60	30	10			280	3,507
800	[75]	65	47	21	8				216	1,803
									1,000	

[a] Reciprocal of dilution.

TABLE 6. *Quantitative correlation of titers in the DT and AG test in 1,000 sera with low antibody titers*

DT titer[a]	AG test titer[a]								Total	Geometric mean titer[a]
	20	40	80	160	320	640	1,280	2,560		
2,560						1	1		2	904
1,280					7	7	[7]	7	28	904
640			1	8	53	[83]	24	5	174	549
320		1	6	41	[117]	51	18	10	244	380
160		8	49	[133]	52	9	3	2	256	169
80	6	31	[98]	32	8	1			176	82
40	21	[56]	17	1	1				96	40
20	[12]	7	3	2					24	34
									1,000	

[a] Reciprocal of dilution.

positive reactions (3, 4) was also disturbing; positive AG test titers have been obtained in sera completely lacking in specific antibodies, as judged by a negative DT and IFA test.

To improve upon this potentially valuable method, the method of antigen preparation described in this report was developed. In attempts to increase the sensitivity of the antigen, it became evident that false-positive reactions are not the exception but the rule; positive results are obtained in the AG test with every serum from normal adults as well as with serum taken from normal infants as early as 6 to 12 months of age (3). The false-positive titer may be high, frequently greater than 1:100 and sometimes 1:1,000 or more. It has previously been shown (3) that this agglutination is due to the binding of "normal" IgM to the surface of the organisms (a phenomenon that is also responsible for the polar staining observed when performing the IFA test [9, 12]). Ablation of IgM activity by the use of 0.2 M 2ME diluted in PBS as a diluent in all the reaction wells proved an effective means of eliminating false-positive reactions. The fact that specific IgM antibodies are inhibited by 2ME is not of practical importance; when present, they are demonstrable in the IgM-IFA test, which should be performed in sera that are positive in the conventional IFA test, DT, or AG test. It has been suggested that the difference in AG test titer before and after 2ME treatment can be used as evidence for the presence of IgM antibody. This method does not allow one to differentiate between agglutination due to "normal" IgM or to "specific" IgM, or to rheumatoid factor, and thus it is not reliable for this purpose.

The AG test is slightly more sensitive than the DT (Table 3), but the two tests are in the same range (approximately 0.1 IU/ml). The tests are equally reproducible (Table 4). Thus, differences in titers observed in individual sera cannot be explained solely by differences in sensitivity of the test, by lack of reproducibility, or by lack of accuracy in dilutions. In some sera, the titer in the DT is higher than that in the AG test, whereas the reverse is observed in many sera. These differences suggest that DT and AG test antibodies are not identical, although they both belong only or mainly to the IgG class. One explanation might be that the AG test measures IgG antibodies belonging to subclasses 1, 2, 3, and also 4. Subclass 4 does not act in the DT since it does not activate the complement system (R. Schreiber and H. A. Feldman, J. Infect. Dis., in press). Whatever the reason, significant differences (more than two serial dilutions) are

observed between DT and AG test titers in some sera. It should be noted that these differences often relate to the length of time the individual has been infected. The AG test titer is most often less than the DT titer during the early weeks of an acute infection (Table 7). The AG test titer is most often slightly greater than the DT titer in older infection and is sometimes much greater in chronic cases. This is frequently substantiated when the evolution of titers in both tests in the same patient is reviewed; the AG test may become positive a few days later than does the DT, and the titer in the AG test often rises more slowly and continues to rise for a few months, during which time the DT titer has stabilized. The AG test titer remains at a higher level for longer periods than does the DT titer. The frequency of this pattern of evolution of DT and AG test titers in individual cases explains the distributions of titers in Tables 4 to 8. However, the reverse pattern is sometimes observed, or titers are found to be identical in the two tests for the entire evolution of the infection. For this reason, differences in DT and AG test titers are never of diagnostic significance in regard to the stage of infection in individual cases. Nevertheless, such differences add to the usefulness of the AG test for the study of paired sera since it is sometimes possible to demonstrate a definite rise in titer with the AG test in sera in which the DT titer is stable after reaching its maximal level. In this regard, the AG test may be useful in the diagnosis of recently acquired infection.

TABLE 7. *Quantitative correlation of titers in the DT and AG test in 200 sera from patients with recent conversion from negative to positive tests and/or rising antibody titers*

DT titer[a]	AG test titer[a]									Total	Geometric mean titer[a]
	<20	20	40	80	160	320	640	1,280	2,560		
2,560						1	2			3	507
1,280					4	16	12	[1]		33	394
640			2	4	11	13	[11]			41	252
320		1	4	13	11	[12]	1			42	135
160		5	6	6	[9]	1				27	70
80	4	11	10	[2]	2					29	<34
40	4	9	[2]	1						16	<25
20	1	[5]	1							7	<22
<20	[1[b]]	1								2	<20
										200	

[a] Reciprocal of dilution.
[b] The IgM-IFA test was the first test to become positive in this previously seronegative patient.

TABLE 8. *Quantitative correlation of titers in the DT and AG test in sera from 202 patients tested in Palo Alto*

DT titer[a]	AG test titer[a]														Total	Geometric mean titer[a]
	<40	40	80	160	320	640	1,280	2,560	5,120	10,240	20,480	40,960	81,920	163,840		
65,536										1			[1]		2	29,000
32,768									1		1	[2]			4	20,000
16,384												1			1	40,000
8,192								1	2		1	3	1	1	9	22,000
4,096						2		1	[1]	3	2	1	1	1	12	10,600
2,048						1	1	[1]	1						4	1,820
1,024							[9]	5	5	5	1				25	3,320
512			1			[5]	7	5	2	1					21	1,400
256			1		[4]	8	6	5	3	4					31	1,380
128				[2]	6	6	5	5		2					26	900
64		1	[2]	6	1	5	2	1			1				19	390
32		[4]	2	3	1	2		1	1						14	186
16		1	1												2	56
8		1	1												2	56
4															0	
<4	29	1													30	
Total	29	8	8	11	12	29	30	25	16	16	6	7	3	2	202	

[a] Reciprocal of dilution.

Differences in the evolution of the DT and AG test antibodies partly explain the discrepancies observed in the screening test (when sera are tested at 1:20 and 1:800). When the discrepancy is a positive DT and negative AG test, in most cases the IgM-IFA test will be positive. This discrepancy is observed in samples obtained during the early days of the infection. The reverse discrepancy (AG test is positive and DT is negative) is most often related to the slight difference in sensitivity of the two tests; in many of these sera, the DT proves to be positive at a dilution of 1:5 or 1:10. But other causes are possible for these discrepancies. For instance, inhibition of complement due to bacterial contamination of the serum sample or to a high anticomplementary activity may lead to a false-negative DT. In a few sera, we felt that the 2ME concentration may have been too low to completely inactivate the effect of the normal IgM at a dilution of 1:20. Finally, in two cases (among 600,000 sera), we have observed a high titer in the AG and IgG-IFA tests, whereas the DT and complement fixation test were negative; this suggested that the IgG antibodies in these two patients might be of restricted heterogeneity and might belong only to the subclass 4 of IgG.

Premarital examination of women for toxoplasmosis is now compulsory in France, and pregnant women with a negative test at the beginning of their pregnancy are further examined every 1 or 2 months up to the time of delivery. To better control the large series of tests that must be performed, it became necessary to perform the serological tests in double series. This is why the modified AG method was introduced as a routine test in one of our laboratories (that of G.D. in Paris) in 1974. During the subsequent 6 years it has proved so simple, useful, and reliable that we felt it would be of interest to others involved in toxoplasma serology.

Since agreement of the AG test with the DT is close to 99%, it would be ideal for use as a screening test for identifying seronegative patients in a population of pregnant women in countries in which the percentage of positive individuals in the age group being screened is relatively low (e.g., United States). This would allow for a rapid and a simple selection of those women who are seropositive and who should be further examined by other serological methods (13; C. B. Wilson and J. S. Remington, submitted for publication). This would also provide a very simple and inexpensive means for the surveillance of seronegative women during pregnancy and for the detection of seroconversions.

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